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Some Reflections on Biological Studies of Needle Diseases[1]

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Biological investigations of several needle diseases are being conducted in widely separated locations. To increase our understanding of the biology of such diseases, results from research conducted in these different locations need to be compared and differences resolved. Presented herein are examples of differences and ways of resolving them with minimal effort, for example, using comparable experimental methods. Comments on the confusion resulting from use of ambiguous terms in specifying needle age are included, and suggestions are offered for clarification.

Keywords: Needle diseases (conifers), tree diseases, pathogen, germination, germ tube.

There has been a rapid increase in the number of investigations of the biology of needle diseases during the last 15 years. These investigations are providing basic information needed for prevention or control of needle diseases by use of genetic resistance or by direct means (fungicides). In many instances, the same diseases are being studied in widely separated locations, particularly those diseases which are economically important. For a more complete understanding of the biology of such diseases, the results from investigations conducted in widely separated locations need to be compared and differences resolved.

There are several obstacles to making needed comparisons, such as differences in the (1) pathogen (strain or species), (2) hosts, and (3) environment. Many differences could be resolved if pathogens from several locations could be used at one or more locations for cross-inoculation studies. Movement of viable fungi for taxonomic studies involving only laboratory work involves low risk of introducing "new" strains to hosts in a given area; however, movement for cross-inoculation purposes has high risk and should be attempted only under certain special conditions.

There is less of a problem in investigating in one location the reaction of hosts from several locations, since hosts can be established in new locations from seed with low risk of introducing pathogens. Actually, the environment may not favor the development of introduced hosts; and even if the new hosts were to develop, the interactions of host and pathogen with environmental factors may result in reactions far different than in locations where the pathogen and hosts are indigenous.

In spite of these serious obstacles, there is considerable comparative work that can be done which will

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increase our knowledge of the biology of needle diseases. For instance, we can evaluate the work of our colleagues and repeat their critical experiments in our locations. Sometimes this can be done with minimum effort. I would like to present some examples.

I reported several years ago that tubes of *Dothistroma pini* conidia were positively directed toward stomata on needles of Austrian and ponderosa pines in eastern Nebraska, U.S.A. (Peterson 1969). Some pathologists investigating infection of Monterey pine by *D. pini* reported that germ tubes were not positively directed toward stomata (Gadgil 1967, Ivory 1972). My information was obtained by observing plastic prints of the surfaces of needles collected in the field. In contrast, observations of germination on Monterey pine needles had been made on needles of seedlings that had been artificially inoculated and then incubated under high humidity. I have found that under such conditions germ tubes of *D. pini* are not positively directed toward stomata on needles of Austrian and ponderosa pines (unpublished data). Possibly, this directed growth of germ tubes is related to a water gradient from stomata to germ tubes and that incubation under high moisture destroys the gradient.

In a recent publication (Setliff and Patton 1974), it was reported that germ tubes of *Scirrhia acicola* conidia were not positively directed toward stomata on Scots pine needles. The authors had observed germination on needles artificially inoculated and incubated under high moisture. In contrast, I have made plastic prints of needles from Scots pine located in a plantation and observed that a high percent of germ tubes were positively directed toward stomata.

We must use artificial conditions in our investigations, but we should try to determine if the phenomena observed under such conditions are comparable to phenomena under natural conditions. Often the differences can be resolved with minimum effort, as is the case in the examples given above.

Spore germination experiments are routinely conducted by most laboratories to determine the effect of temperature on germination. Two of the most commonly used methods involve germination in water (Van Tieghem cells) or germination on solid media such as water agar. Investigators seldom conduct tests using both liquid (water) and solid media. Thus, in comparing germination results, it frequently is not possible to determine whether apparent differences in results are related to methods or to different "strains" of the fungus. Again, this problem can be resolved with minimum effort.

Most reports on spore germination do not include data on germ tube growth, but such data would increase considerably our knowledge of pathogens. For example, percent germination for many pathogens is near optimum over a relatively wide temperature range, but germ tube growth is usually near optimum over a much narrower temperature range.

The standardization of methods for producing spores for inoculations is essential when a series of experiments is to be conducted. Thus when the cultures from which spores are obtained for a series of experiments include a broad range of ages (3 to 6 weeks, for example), care must be taken to see that culture age does not have a significant influence on germination. Even slight differences in culture age may cause large differences in germination. In work with *Cercospora sequoiae* var. *juniperi* on *Juniperus* species, we found striking differences in percent germination and germ tube lengths of spores from 7- to 14-day-old (carrot-leaf decoction agar) cultures.[3] After incubation on water agar for 24 hours at 24°C, percent germination and germ tube lengths for spores from 7-day-old cultures were 91 percent and 48 micrometers, for 10-day-old cultures 57 percent and 22 micrometers, and for 14-day-old cultures 16 percent and 10 micrometers.

Needle age is not adequately specified by some investigators of needle diseases. Those concerned with the biology of needle diseases usually include information on needle age in their reports; however, the terminology is often confusing. Terms such as **one-year-old, two-year-old** are frequently used in the literature from English-speaking countries. In most papers when one-year-old is used, needles of the second year are being referred to; but in others, reference is actually being made to **current-year** needles. I do not recommend standardization of terms for use in specifying needle age, since no system of terms would be adequate for all situations. I suggest the discontinuance of the use of one-year-old and two-year-old when referring to age of needles on trees in stands. I recommend the use of **first-year** and **second-year** in specifying needle age, but it must be indicated that **first-year** refers to **current-year.**

Mycologists studying needle-inhabiting fungi can apparently achieve their taxonomic objectives without reference to needle age, since seldom do their publications include information on age of needles. In his first major treatment of the Hypodermataceae, Darker (1932) included some information on needle age; but in later treatments he did not. I suggest that, when practical, those working on taxonomic aspects (mycologists, pathologists) include in their publications information on needle age, even if the information is limited.

[3] *Peterson, Glenn W. Epidemiology and control of a blight of* Juniperus virginiana *caused by* Cercospora sequoiae *var.* juniperi. (*Manuscript accepted for Phytopathology.*)

Pathologists working on the biology of needle diseases could in a similar way be more helpful to taxonomists if they would include in their publications information needed by taxonomists, such as dimensions and other characteristics of spores and fruiting bodies.

The incubation period (time between infection and first appearance of symptoms) is rather long for many needle diseases. This presents particularly difficult problems in determining when initial infection occurs. Information on when spores are first produced and dispersed may provide leads; however, there can be lengthy intervals between spore production and spore dispersal, and between spore dispersal and initial infection. Thus this type of information cannot provide conclusive information on time of initial infection.

If symptoms and fruiting bodies develop early in the growing season on current-year needles, rather accurate estimates can be made of the time of initial infection. Thus the determination of conditions under which infection can occur is simplified. If current-year needles are initially resistant to infection, as with *Dothistroma pini* on Austrian and ponderosa pine in eastern Nebraska, then indirect means will usually be needed to determine time of initial infection. In this case, time of initial infection was determined by leaving needles unprotected (not sprayed with fungicide) for specific periods of time, then protecting them with fungicide (Peterson 1965). Such tests are simple because several treatments can be made on a single tree, since only a few shoots need to receive the same treatment. In this manner information on variation in resistance among trees can be accumulated rapidly and with precision. Others have approached this problem by placing seedlings within infected stands for specific time periods, then removing them and observing for evidence of infection. This method can be effective, but caution should be exercised to assure that differences in susceptibility between seedlings and the trees in the stands do not confound results.

Incubation periods determined by artificial inoculation of seedlings may be useful for field determinations of initial infection. However, the incubation periods determined by artificial methods are usually much shorter than those in the field. For example, Gadgil (1974) in a fine series of experiments on *Dothistroma pini* infection of inoculated Monterey pine seedlings, obtained incubation periods of less than 2 weeks; however, in the field in New Zealand the incubation period on Monterey pine varies from 5 to 16 weeks (Gilmour 1967).

Determining when infection occurs throughout the growing season is more difficult than determining when initial infection occurs. Some investigators have approached this problem by placing bags over shoots prior to spore dispersal, removing the bags for a short period to expose needles to spores, and then replacing bags (or protecting shoots with fungicide). We have not been able to use this method because high temperatures within the bags had an adverse effect on shoots and needles. Furthermore, we found that when the shoots of ponderosa and Austrian pines were bagged, infection of the current-year needles occurred earlier than usual. We thought that this early breakdown in resistance of current-year needles might be due to the effect bagging had on needle surface waxes. To explore this possibility, we washed needles with wax solvents; however, the washed needles retained their resistance. We next sought to determine if the amount of surface wax on current-year needles in mid-July (when the needles became susceptible) differed from amounts present on needles earlier in the growing season (when needles were resistant). We did not find any differences in wax amounts that were correlated with time of change of needles from resistant to susceptible.[4] The methods of Schütt (1971) and Schuck (1972) should be used to determine if there are changes in the nature of the waxes during the growing season that might be correlated with change in resistance.

Those working on needle diseases are well aware that the amount of infection in one year may not provide information on the amount of infection the next year. Our first work on Dothistroma blight in eastern Nebraska included the installation of a control test. The year prior to the test, infection had been at a very high level in the plantation; but in 1959 there was essentially no infection; thus no information on control was obtained. During this test, I obtained data from a U.S. Weather Bureau station located 8 kilometers from the test site. A check of the weather records did not reveal an obvious reason for the lack of infection. Nor was information on the number of spores being dispersed sufficient to explain the lack of infection. It was at this point that I decided in subsequent work to periodically make plastic leaf prints of needles to determine what was happening to the spores on the needle surfaces. Through this means we were able to determine those periods in which high numbers of spores were dispersed but in which spores did not germinate on needles (Peterson 1973).

Usually several years' observations are needed to get a clear picture of fungus and disease development in the field (Lanier and Sylvestre 1971, Peterson 1973). The incubation period of *Dothistroma pini* is much shorter in western Oregon, than in the central

[4] *Walla, James A., and Glenn W. Peterson.* Dothistroma pini *and* Diplodia pinea *not affected by surface wax of pine needles. (Manuscript accepted for Plant Dis. Rep.)*

United States.[5] Though fruiting bodies may form on needles during the year of infection in eastern Nebraska, we rarely find conidia in these fruiting bodies until the following spring (April, May). In contrast, in Oregon conidia are frequently produced on needles infected during the current year. Are these differences due to "strains" of the fungus, different weather conditions, or both? There are differences in morphology between conidia from the central United States and the western United States (Funk and Parker 1966, Thyr and Shaw 1964). Certainly the weather is different. We have some suggestive evidence obtained from work in both areas.[5] In the central United States, the presence of conidia in the year of infection has occurred only during years when weather late in the growing season was unusually warm, but during which there were a few nights when temperatures were unusually low. Is the short incubation period in the West due to the lower nighttime temperatures which occur frequently there? We have a suggestion that this is the case, but are not certain.

Again this brings us back to the point made earlier, that for more rapid progress in the investigations of the biology of needle disease, we should resolve the differences in results obtained from different locations. Furthermore, we should examine some of the newer methods being used by some investigators and, where possible, use these methods in our locations. By so doing, we can more quickly explain phenomena associated with needle diseases and more quickly formulate principles that can be utilized in preventing or controlling needle diseases.

[5] *Peterson, Glenn W., and George M. Harvey. Dispersal of* Scirrhia (Dothistroma) pini *conidia and disease development in a shore pine plantation in western Oregon. (Manuscript accepted for Plant Dis. Rep.)*

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